#4

AMENDMENT

4/8

In the specification:

Please replace the paragraph beginning at page 95, line 1, with the following rewritten paragraph:

B1

The present invention also provides expression vectors in which the recombinant PKS genes of the invention are under the control of a *Myxococcus xanthus* promoter. To construct an illustrative vector, the promoter of the *pilA* gene of *M. xanthus* was isolated as a PCR amplification product. Plasmid pSWU357, which comprises the *pilA* gene promoter and is described in Wu and Kaiser, Dec. 1997, *J. Bact. 179*(24):7748-7758, was mixed with PCR primers Seq1 and Mxpil1 primers:

Seq1: 5'-AGCGGATAACAATTTCACACAGGAAACAGC-3' (SEQ ID NO:1); and

Mxpil1: 5'-TTAATTAAGAGAAGGTTGCAACGGGGGGC-3' (SEQ ID NO:2),

and amplified using standard PCR conditions to yield an ~800 bp fragment. This fragment was cleaved with restriction enzyme *KpnI* and ligated to the large *KpnI-EcoRV* restriction fragment of commercially available plasmid pLitmus 28 (New England Biolabs). The resulting circular DNA was designated plasmid pKOS35-71B.

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Please replace the paragraph beginning at page 96, line 4, with the following rewritten paragraph:

B2

The sequence of the *pilA* promoter in these plasmids is shown below (SEQ ID NO:3).

CGACGCAGGTGAAGCTGCTTCGTGTGCTCCAGGAGCGGAAGGTGAAGCCGGTCGGCAG
CGCCGCGGAGATTCCCTTCCAGGCGCGTGTCATCGCGGCAACGAACCGGCGGCTCGAA
GCCGAAGTAAAGGCCGGACGCTTTCGTGAGGACCTCTTCTACCGGCTCAACGTCATCA
CGTTGGAGCTGCCTCCACTGCGCGAGCGTTCCGGCGACGTGTCGTTGCTGGCGAACTAC
TTCCTGTCCAGACTGTCGGAGGAGTTGGGGCGACCCGGTCTGCGTTTCTCCCCCGAGAC
ACTGGGGCTATTGGAGCGCTATCCCTTCCCAGGCAACGTGCGGCAGCTGCAGAACATG
GTGGAGCGGGCCGCGACCCTGTCGGATTCAGACCTCCTGGGGCCCTCCACGCTTCCACC

CGCAGTGCGGGGCGATACAGACCCCGCCGTGCGTCCCGTGGAGGGCAGTGAGCCAGG
GCTGGTGGCGGGCTTCAACCTGGAGCGGCATCTCGACGACAGCGAGCGGCGCTATCTC
GTCGCGGCGATGAAGCAGGCCGGGGGCGTGAAGACCCGTGCTGCGGAGTTGCTGGGCC
TTTCGTTCCGTTCATTCCGCTACCGGTTGGCCAAGCATGGGCTGACGGATGACTTGGAG
CCCGGGAGCGCTTCGGATGCGTAGGCTGATCGACAGTTATCGTCAGCGTCACTGCCGA
ATTTTGTCAGCCCTGGACCCATCCTCGCCGAGGGGATTGTTCCAAGCCTTGAGAATTGG
GGGGCTTGGAGTGCGCACCTGGGTTGGCATGCGTAGTGCTAATCCCATCCGCGGGCGC
AGTGCCCCCCGTTGCAACCTTCTCTTAATTAA

B2 Cont

Please replace the paragraph beginning at page 147, line 19, with the following rewritten paragraph:

To increase the levels of phosphopantetheinyl transferase (PPTase) protein, the PPTase from *Stigmatella aurantiaca* strain DW4 can be added to K111-32.25. This is done by PCR amplification of *mtaA* from DW4 chromosomal DNA using the primers 111-44.1 (AAAAGCTTCGGGGCACCTCCTGGCTGTCGGC) (SEQ ID NO:4) and 111-44.4 (GGTTAATTAATCACCCTCCTCCCACCCCGGGCAT) (SEQ ID NO:5). See Silakowski *et al.*, 1999, *J. Biol. Chem. 274*(52):37391-37399, incorporated herein by reference. The ~800 bp fragment was cleaved with *Nco*I and ligated into the pUHE24-2B that had been cleaved with *Pst*I, the DNA ends made blunt with the Klenow fragment of DNA polymerase I, and cleaved with *Nco*I. This plasmid is designated pKOS111-54. The *mtaA* gene is transfered to plasmid pKOS35-82.1, which contains the tetracycline resistance conferring gene, the Mx8 att site and the *Myxococcus xanthus pilA* promoter to drive expression of *mtaA*. This plasmid is introduced into *M. xanthus* and integrated into the Mx8 phage attachment site.

B3

Please replace the paragraph beginning at page 148, line 22, with the following rewritten paragraph:

Primers 90-66.1 and 90-67 (shown below) were used to clone the upstream flanking region. Primer 90-67 is at the 5' end of the PCR fragment and 90-66.1 is at the 3' end of the PCR

B4

fragment. The fragment ends 2481 bp before the start codon for the *epoA* gene. The ~2.2 kb fragment was cut with *Hind*III. Klenow polymerase was added to blunt the *Hind*III site. This fragment was ligated into the *Hinc*II site of pNEB193. Clones with the proper orientation, those with the *EcoR*I site at the downstream end of the insert and *Hind*III at the upstream end of the insert, were selected and named pKOS90-90.

B4 Cont

90-66.1: 5' GCGGG AAGCTT TCACGGCGCAGGCCCTCGTGGG 3' (SEQ ID NO:6)
linker *HindIII* primer

90-67: 5' GC GGTACC TTCAACAGGCAGGCCGTCTCATG 3' (SEQ ID NO:7)
linker *KpnI* primer--

Please replace the paragraph beginning at page 149, line 29, with the following rewritten paragraph:

B5 The myxothiazol promoter was PCR amplified from Stigmatella aurantiaca chromosomal DNA (strain DW4) using primers 111-44.3 and 111-44.5 (shown below). The ~554 bp band was cloned into the HincII site of pNEB193 to create pKOS90-107. Plasmid pKOS90-107 was cut with PstI and XbaI and Klenow filled-in. The 560 bp band was cloned into pKOS90-102 and pKOS90-106 cut with PacI and Klenow filled-in (PacI cuts only once in pKOS90-102 and pKOS90-106). Plasmids were screened for the correct orientation. The MTA promoter/pKOS90-102 plasmid was named pKOS90-114 (13.36 kb) and MTA promoter/pKOS90-106 plasmid was named pKOS90-113 (13.26 kb).

111-44.3 5' AA AAGCTT AGGCGGTATTGCTTTCGTTGCACT 3' (SEQ ID NO:8)
linker HindIII primer

111-44.5 5' GG TTAATTAAGGTCAGCACACGGTCCGTGTGCAT 3' (SEQ ID NO:9)
linker PacI primer--

Please replace the paragraph beginning at page 150, line 22, with the following rewritten paragraph:

The putative promoter for TA along with *taA*, which encodes a putative transcriptional anti-terminator, was PCR amplified from strain TA using primers 111-44.8 (AAAGATCTCTCCCGATGCGGGAAGGC) (SEQ ID NO:10) and 111-44.9 (GGGGATCCAATGGAAGGGGATGTCCGCGGAA) (SEQ ID NO:11). The ca. 1.1 kb fragment was cleaved with *Bam*HI and *Bgl*II and ligated into pNEB193 cleaved with *Bam*HI.This plasmid is designated pKOS111-56.1. The plasmid pKOS111-56.1 was cut with *Eco*RI and *Hin*dIII and Klenow filled-in. The ~1.1 kb band was cloned into pKOS90-102 and pKOS90-106 cut with *Pac*I and Klenow filled-in (*Pac*I cuts only once in pKOS90-102 and pKOS90-106). Plasmids were screened for the correct orientation. The TA promoter/90-102 plasmid was named pKOS90-115 (13.9kb), and the TA promoter/pKOS90-106 plasmid was named pKOS90-111 (13.8kb).

Please replace the paragraph beginning at page 152, line 17, with the following rewritten paragraph:

These plasmids are electroporated into *Myxococcus* host cells containing the epothilone PKS genes, and kanamycin resistant transformants selected to identify the single crossover recombinants. These transformants are selected for galactose resistance to identify the double crossover recombinants, which are screened by Southern analysis and PCR to identify those containing the desired recombination event. The desired recombinants are grown and tested for epothilone production.

111-44.6 5' GG TTAATTAACATCGCGCTATCAGCAGCGCTGAG3' (SEQ ID NO:12)
linker PacI primer

111-44.7 5' GG TTAATTAA TCCTCAGCGGCTGACCCGCTCGCG3' (SEQ ID NO:13)
linker PacI primer

Please replace the paragraph beginning at page 153, line 9, with the following rewritten paragraph:

The downstream flanking region of the epothilone PKS gene was PCR amplified using primers 90-103 (5'-AAAAAATGCATCTACCTCGCTCGTGGCGGTT-3') (SEQ ID NO:14) and 90-107.1 (5'-CCCCC TCTAGA ATAGGTCGGCAGCGGTACCCG-3') (SEQ ID NO:15) from plasmid pKOS35-78.2. The ~2 kb PCR product was cut with *NsiI/XbaI* and ligated with pSL1190 digested with *NsiI* and *SpeI* to create pKOS90-123 (~5.4 kb). A ~2 kb PCR fragment amplified with primers 90-105 (5'-TTTTTATGCATGCGGCAGTTTGAACGG-AGATGCT-3') (SEQ ID NO:16) and 90-106 (5'-CCCCCGAATTCTCCCGGAAGGCACACGGAGAC-3') (SEQ ID NO:17) from pKOS35-78.2 DNA was cut with *NsiI* and ligated with pKOS90-123 digested with *NsiI/EcoRV* to create pKOS90-130 (~7.5 kb). When this plasmid is cut with *NsiI*, and the DNA ends made blunt with the Klenow fragment of DNA polymerase I and religated, plasmid pKOS90-131 is created. To clone the *galK*/kan^r cassette into this plasmid, plasmid KG-2 is cut with *BamHI/NdeI* and made blunt with the Klenow fragment of DNA polymerase I. The 3 kb fragment is cloned into the *DraI* site of pKOS90-131 (*DraI* cuts three times in the vector) to create plasmid pKOS90-132 (10.5 kb). The *NsiI* site is used for the purpose of creating the desired change from cysteine to alanine to effect the KS2 knockout. When pKOS90-130 is cut with *NsiI*, made blunt with the Klenow fragment from DNA polymerase I and re-ligated, the codon for cysteine is replaced with a codon for alanine. The resulting plasmid can be introduced into *Myxococcus xanthus* strains of the invention in accordance with the protocols described above to create the desired strains.

B8

Please replace the paragraph beginning at page 160, line 8, with the following rewritten paragraph:

Inactivation of the KR domain in extender moduler 4 of the epothilone PKS results in a hybrid PKS of the invention useful in the production of 13-keto epothilones. The extender module 4 KR domain was modified by replacing the wild-type gene with various deleted versions as described below. First, fragments were amplified using plasmid pKOS39-118B (a subclone of the *epoD* gene from cosmid pKOS35-70.4) as a template. The oligonucleotide primers for forming the left side of the deletion were TL3 and TL4, shown below:

B9

TL3: 5'-AT*GAATTC*ATGATGGCCCGAGCAGCG (SEQ ID NO:18); and

TL4: 5'-AT*CTGCAG*CCAGTACCGCTGCCGCTGCC (SEQ ID NO:19).

The oligonucleotide primers for forming the right side of the deletion were TL5 and TL6, shown below:

TL5: 5'-GC*TCTAGA*ACCCGGAACTGGCGTGGCCTGT (SEQ ID NO:20); and

TL6: 5-GC*AGATCT*ACCGCGTGAGGACACGGCCTT (SEQ ID NO:21).--

Please replace the paragraph beginning at page 160, line 22, with the following rewritten paragraph:

--The PCR fragments were cloned into vector Litmus 39 and sequenced to verify that the desired fragments were obtained. Then, the clone containing the TL3/TL4 fragment was digested with restriction enzymes *Pst*I and *Bam*HI, and the ~ 4.6 kb fragment was isolated. The 2.0 kb PCR fragment obtained using primers TL5/TL6 was treated with restriction enzymes *Bgl*II and *Xba*I and then ligated to either (i) the "short" KR linkers TL23 and TL24 (that are annealed together to form a double-stranded linker with single-stranded overhangs) to yield pKOS122-29; or (ii) the "long" (epoDH3*) linker, obtained by PCR using primers TL33+TL34 and then treatment with restriction enzymes *Nsi*I and *Spe*I, to yield plasmid pKOS122-30. The sequences of these oligonucleotide linkers and primers are shown below:

TL23: 5'-GGCGCCGGCCAAGAGCGCCGCGCCGGTCGGCGGGCCAGCCGGGGACGGGT (SEQ ID NO:22);

TL24: 5'-CTAGACCCGTCCCCGGCTGGCCCGCCGACCGGCGCGGCGCTCTTGGCCG-GCGCCTGCA (SEQ ID NO:23);

TL33: 5'-GG*ATGCAT*GCGCCGGCCGAAGGGCTCGGA (SEQ ID NO:24); and

TL34: 5'-TC*ACTAGT*CAGCGACACCGGCGCTGCGTTT (SEQ ID NO:25).--

Please replace the paragraph beginning at page 162, line 21, with the following rewritten paragraph:

--Replacement of the extender module 5 KR, DH, and ER domains of the epothilone PKS with a heterologous KR domain, such as the KR domain from extender module 2 of the rapamycin PKS or extender module 3 of the FK520 PKS, results in a hybrid PKS of the invention useful in the production of 13-hydroxy epothilones. This construction is carried out in a manner similar to that described in part A of this example. The oligonucleotide primers for amplifying the desired portions of the *epoD* gene, using plasmid pKOS39-118B as a template, were:

TL7: 5'-GCG*CTCGAG*AGCGCGGGTATCGCT (SEQ ID NO:26);

B11 cont

TL8: 5'-GAG*ATGCAT*CCAATGGCGCTCACGCT (SEQ ID NO:27);

TL9: 5'-GC*TCTAGA*GCCGCGCGCCTTGGGGCGCT (SEQ ID NO:28); and

TL10: 5-GC*AGATCT*TGGGGCGCTGCCTGTGGAA (SEQ ID NO:29).--

Please replace the paragraph beginning at page 163, line 5, with the following rewritten paragraph:

--The PCR fragment generated from primers TL7/TL8 was cloned into vector LITMUS 28, and the resulting clone was digested with restriction enzymes *Nsi*I and *Bgl*II, and the 5.1 kb fragment was isolated and ligated with the 2.2 kb PCR fragment generated from TL9/TL10 treated with restriction enzymes *Bgl*II and *Xba*I and ligated to the KR cassettes. The KR cassette from the FK520 PKS was generated by PCR using primers TL31 and TL32 and then digestion with restriction enzymes *Xba*I and *Pst*I. These primers are shown below:

B12

TL31: 5'-GG*CTGCAG*ACCCAGACCGCGGGCGACGC (SEQ ID NO:30); and

TL32: 5'-GC*TCTAGA*GGTGGCGCCGGCCGCCCGGCG (SEQ ID NO:31).--

Please replace the paragraph beginning at page 164, line 7, with the following rewritten paragraph:

--Inactivation of the KR domain of extender module 6 of the epothilone PKS results in a novel PKS of the invention capable of producing the 9-keto-epothilones. The KR domain can be inactivated by site-specific mutagenesis by altering one or more conserved residues. The DNA and amino acid sequence of the KR domain of extender module 6 of the epothilone PKS is shown below:

B13

```
 36710       36720       36730       36740       36750
GACGGCACCTACCTCGTGACCGGCGGTCTGGGTGGGCTCGGTCTGA
 D  G  T  Y  L  V  T  G  G  L  G  G  L  G  L>
      36760       36770       36780       36790       36800
GCGTGGCTGGATGGCTGGCCGAGCAGGGGGCTGGGCATCTGGTGCTGGTG
S  V  A  G  W  L  A  E  Q  G  A  G  H  L  V  L  V>
      36810       36820       36830       36840       36850
GGCCGCTCCGGTGCGGTGAGCGCGGAGCAGCAGACGGCTGTCGCCGCGCT
 G  R  S  G  A  V  S  A  E  Q  Q  T  A  V  A  A  L>
      36860       36870       36880       36890       36900
```

```
CGAGGCGCACGGCGCGCGTGTCACGGTAGCGAGGGCAGACGTCGCCGATC
  E  A  H  G  A  R  V  T  V  A  R  A  D  V  A  D>
     36910      36920      36930      36940      36950
GGGCGCAGATCGAGCGGATCCTCCGCGAGGTTACCGCGTCGGGGATGCCG
R  A  Q  I  E  R  I  L  R  E  V  T  A  S  G  M  P>
     36960      36970      36980      36990      37000
CTCCGCGGCGTCGTTCATGCGGCCGGTATCCTGGACGACGGGCTGCTGAT
 L  R  G  V  V  H  A  A  G  I  L  D  D  G  L  L  M>
     37010      37020      37030      37040      37050
GCAGCAAACCCCCGCGCGGTTCCGCGCGGTCATGGCGCCCAAGGTCCGAG
  Q  Q  T  P  A  R  F  R  A  V  M  A  P  K  V  R>
     37060      37070      37080      37090      37100
GGGCCTTGCACCTGCATGCGTTGACACGCGAAGCGCCGCTCTCCTTCTTC
G  A  L  H  L  H  A  L  T  R  E  A  P  L  S  F  F>
     37110      37120      37130      37140      37150
GTGCTGTACGCTTCGGGAGCAGGGCTCTTGGGCTCGCCGGGCCAGGGCAA
 V  L  Y  A  S  G  A  G  L  L  G  S  P  G  Q  G  N>
     37160      37170      37180      37190      37200
CTACGCCGCGGCCAACACGTTCCTCGACGCTCTGGCACACCACCGGAGGG
  Y  A  A  A  N  T  F  L  D  A  L  A  H  H  R  R>
     37210      37220      37230      37240      37250
CGCAGGGGCTGCCAGCATTGAGCATCGACTGGGGCCTGTTCGCGGACGTG
A  Q  G  L  P  A  L  S  I  D  W  G  L  F  A  D  V>
GGTTTG (SEQ ID NO:32)
 G  L> (SEQ ID NO:33)
```

B13 Cont

Please replace the paragraph beginning at page 165, line 16, with the following rewritten paragraph:

The DNA and amino acid sequence of the mutated and inactive KR domain of extender module 6 of the novel 9-keto-epothilone PKS provided by the present invention is shown below:

```
 36710      36720      36730      36740      36750
GACGGCACCTACCTCGTGACCGGCGCTCTGGGTGGGCTCGGTCTGA
 D  G  T  Y  L  V  T  G  A  L  G  G  L  G  L>
     36760      36770      36780      36790      36800
GCGTGGCTGGATGGCTGGCCGAGCAGGGGGCTGGGCATCTGGTGCTGGTG
S  V  A  G  W  L  A  E  Q  G  A  G  H  L  V  L  V>
     36810      36820      36830      36840      36850
GGCCGCTCCGGTGCGGTGAGCGCGGAGCAGCAGACGGCTGTCGCCGCGCT
 G  R  S  G  A  V  S  A  E  Q  Q  T  A  V  A  A  L>
     36860      36870      36880      36890      36900
CGAGGCGCACGGCGCGCGTGTCACGGTAGCGAGGGCAGACGTCGCCGATC
  E  A  H  G  A  R  V  T  V  A  R  A  D  V  A  D>
     36910      36920      36930      36940      36950
GGGCGCAGATCGAGCGGATCCTCCGCGAGGTTACCGCGTCGGGGATGCCG
R  A  Q  I  E  R  I  L  R  E  V  T  A  S  G  M  P>
     36960      36970      36980      36990      37000
CTCCGCGGCGTCGTTCATGCGGCCGGTATCCTGGACGACGGGCTGCTGAT
 L  R  G  V  V  H  A  A  G  I  L  D  D  G  L  L  M>
     37010      37020      37030      37040      37050
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B14

```
GCAGCAAACCCCCGCGCGGTTCCGCGCGGTCATGGCGCCCAAGGTCCGAG
  Q  Q  T  P  A  R  F  R  A  V  M  A  P  K  V  R>
     37060      37070      37080      37090      37100
GGGCCTTGCACCTGCATGCGTTGACACGCGAAGCGCCGCTCTCCTTCTTC
G  A  L  H  L  H  A  L  T  R  E  A  P  L  S  F  F>
     37110      37120      37130      37140      37150
GTGCTGTACGCTTCGGGAGCAGGGCTCTTGGGCTCGCCGGGCCAGGGCAA
 V  L  Y  A  S  G  A  G  L  L  G  S  P  G  Q  G  N>
     37160      37170      37180      37190      37200
CTTCGCCACGGCCAACACGTTCCTCGACGCTCTGGCACACCACCGGAGGG
  F  A  T  A  N  T  F  L  D  A  L  A  H  H  R  R>
     37210      37220      37230      37240      37250
CGCAGGGGCTGCCAGCATTGAGCATCGACTGGGGCCTGTTCGCGGACGTG
A  Q  G  L  P  A  L  S  I  D  W  G  L  F  A  D  V>
GGTTTG (SEQ ID NO:34)
G  L>  (SEQ ID NO:35)--
```

B14 Cont

Please replace the paragraph beginning at page 168, line 1, with the following rewritten paragraph:

A first PCR is used to generate an ~1.6 kb fragment from pKOS39-125 DNA used as template. The PCR fragment is subcloned into vector LITMUS28 at the *Hin*dIII and *Bgl*II sites and sequenced; a plasmid with the desired sequence is designated P1. The oligonucleotides used in this PCR are:

B15

TLII-1: 5'-ACAAGCTTGCGAAAAAGAACGCGTCT (SEQ ID NO:36) ; and

TLII-2: 5'-CGAGATCTGCCGGGCGAGGAAGCGGCCCTG (SEQ ID NO:37).

Please replace the paragraph beginning at page 168, line 8, with the following rewritten paragraph:

A second PCR is used to generate an ~1.9 kb fragment using pKOS39-125 DNA as template. The PCR fragment is subcloned into vector LITMUS28 at the *Nsi*I and *Spe*I sites and sequenced; a plasmid with the desired sequence is designated P2. The oligonucleotides used in this PCR are:

B16

TLII-3B: 5'-GCATGCATGCGCCGGTCGATGGTGAG SEQ ID NO:38); and

TLII-4: 5'-AGACTAGTCACCGGCTGGCCCACCACAAGG (SEQ ID NO:39).

Please replace the paragraph beginning at page 168, line 15, with the following rewritten paragraph:

Plasmid P1 is then digested with restriction enzymes *Bgl*II and *Spe*I, and the 4.5 kb fragment is isolated and ligated with the ~1.9 kb *Nsi*I-*Spe*I restriction fragment from plasmid P2 and with one of the three replacement AT fragments (FKAT2, epoAT2, tmbAT3) isolated as *Nsi*I-*Bgl*II restriction fragments to obtain plasmids P3.1, P3.2, and P3.3. The replacement AT fragments are generated by PCR using the following oligonucleotide primers:

for FKAT2:

TLII-20: 5'-GCATGCATCCAGTAGCGGTCACGGCGGA (SEQ ID NO:40); and

TLII-21: 5'-CGAGATCTGTGTTCGCGTTCCCCGGGCAG (SEQ ID NO:41);

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for tmbAT3:

TLII-13: 5'-GCATGCATCCAGTAGCGCTGCCGCTGGAAT (SEQ ID NO:42); and

TLII-14: 5'-GCAGATCTGTGTTCGTGTTCCCCGGCCA (SEQ ID NO:43); and

for epoAT2:

TLII-17: 5'-GCATGCATCCAGTACCGCTCGCGCTG (SEQ ID NO:44); and

TLII-18: 5'-CGAGATCTGTCTTCGTCTTTCCCGGCCAG (SEQ ID NO:45).

Please replace the paragraph beginning at page 170, line 4, with the following rewritten paragraph:

A first PCR is used to generate an ~1.8 kb fragment from pKOS39-125 DNA used as template. The PCR fragment is subcloned into vector LITMUS28 at the *Nsi*I and *Spe*I sites and sequenced; a plasmid with the desired sequence is designated P4. The oligonucleotides used in this PCR are:

B18

TLII-5: 5'-GGATGCATGTCGAGCCTGACGCCCGCCG (SEQ ID NO:46); and

TLII-6: 5'-GCACTAGTGATGGCGATCTCGTCATCCGCCGCCAC (SEQ ID NO:47).

Please replace the paragraph beginning at page 170, line 11, with the following rewritten paragraph:

A second PCR is used to generate an ~2.1 kb fragment using pKOS039-118B DNA as template. The oligonucleotides used in this PCR are:

TL16: ACAGATCTCGGCGCGCTGCCGCCGGAG (SEQ ID NO:48); and

TL15: GGTCTAGACTCGAACGGCTCGCCACCGC (SEQ ID NO:49).

Please replace the paragraph beginning at page 170, line 16, with the following rewritten paragraph:

The PCR fragment is subcloned into LITMUS 28 at the *Eco*RV restriction site, and a plasmid with the desired sequence is identified by sequencing and designated as plasmid pKOS122-4. Plasmid pKOS122-4 is then digested with restriction enzymes *Bgl*II and *Spe*I, and the 4.8 kb fragment is isolated and ligated with the ~1.8 kb *Nsi*I-*Spe*I restriction fragment from plasmid P4 and with one of the three replacement AT fragments (FKAT3, epoAT5, tmbAT4) isolated as *Nsi*I-*Bgl*II restriction fragments to obtain plasmids P5.1, P5.2, and P5.3. The replacement AT fragments are generated by PCR using the following oligonucleotide primers:

for FKAT3:

TLII-11: 5'-GTATGCATCCAGTAGCGGACCCGCTCGA (SEQ ID NO:50); and

TLII-12: 5'-GCAGATCTGTGTGGCTCTTCTCCGGACA (SEQ ID NO:51);

for tmbAT4:

TLII-15; 5'-GCATGCATCCAGTAGCGCTGCCGCTGGAAC (SEQ ID NO:52); and

TLII-16; 5'-GGAGATCTGCGGTGCTGTTCACGGGGCA (SEQ ID NO:53); and

for PCR epoAT5:

TLII-19; 5'-GTAGATCTGCTTTCCTGTTCACCGGACA (SEQ ID NO:54); and

TL8 (see part B of this Example).

Please replace the paragraph beginning at page 172, line 6, with the following rewritten paragraph:

The PCR fragment generated from primers TL11 and TL12 using plasmid pKOS39-118B as a template is cloned into vector LITMUS 28. The PCR primers used are:

TL11: 5'-GGATGCATCTCACCCCGCGAAGCG (SEQ ID NO:55); and

B21 Cont

TL12: 5'-GTACTAGTCAAGGGCGCTGCGGAGG (SEQ ID NO:56).

Please replace the paragraph beginning at page 173, line 18, with the following rewritten paragraph:

A first PCR is used to generate an ~1.8 kb fragment from pKOS39-124 DNA used as template. The PCR fragment is subcloned into vector LITMUS28 at the *Xba*I and *Bgl*II sites and sequenced; a plasmid with the desired sequence is designated P9. The oligonucleotides used in this PCR are:

B22

TLII-7: 5'-GCAGATCTGCCGCGCGAGGAGCTCGCGAT (SEQ ID NO:57); and

TLII-8: 5'-CATCTAGAGCCGCTCCTGTGGAGTCAC (SEQ ID NO:58).

Please replace the paragraph beginning at page 174, line 1, with the following rewritten paragraph:

A second PCR is used to generate an ~1.9 kb fragment using pKOS39-124 DNA used as template. The PCR fragment is subcloned into vector LITMUS28 at the *Nsi*I and *Spe*I sites and sequenced; a plasmid with the desired sequence is designated P10. The oligonucleotides used in this PCR are:

B23

TLII-9B: 5'-GGATGCATGCGCCGGCCGAAGGGCTCGGAG (SEQ ID NO:59); and

TLII-10: 5'-GCACTAGTGATGGCGATCGGGTCCTCTGTCGC (SEQ ID NO:60).

Please replace the paragraph beginning at page 175, line 14, with the following rewritten paragraph:

In one embodiment, a strain that produces 10, 11-dehydroepothilone D is constructed by inactivating the enoyl reductase (ER) domain of extender module 5. In one embodiment, the ER inactivation is accomplished by changing the two glycines (-Gly-Gly-) in the NADPH binding region to an alanine and serine (-Ala-Ser-). The 2.5 kb BbvCI-HindIII fragment from plasmid pKOS39-118B (a subclone of the *epoD* gene from cosmid pKOS35-70.4) has been cloned into

B24

pLitmus28 as pTL7 which is used as a template for site directed mutagenesis. The oligonucleotide primers for introducing the –Gly-Gly- to –Ala-Ser- mutations into the NADPH binding domain are:

TLII-22, 5'-TGATCCATGCTGCGGCCG***CTA***GCGTGGGCATGGCCGC (SEQ ID NO:61).

TLII-23, 5'-GCGGCCATGCCCACGC***TAG***CGGCCGCAGCATGGATCA (SEQ ID NO:62).